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The main purpose of our work is to understand the role of Foxm1 in the development of prostate cancer, provide information about molecular mechanisms whereby Foxm1 controls epithelial cell proliferation during PCa, and determine whether the inhibition of Foxm1 may potentially be beneficial during PCa therapy, therefore leading to the development of the novel therapeutic target for prostate cancer chemotherapy.
During the second year of grant support, we demonstrated that the deletion of Foxm1 from prostate epithelial cells reduced prostate carcinogenesis in mouse model. Decreased carcinogenesis in epFoxm1-/-/ TRAMP mice was associated with decreased proliferation of tumor cells and reduced expression of cell cycle regulatory genes Cdc25b, Cyclin B1, and Plk-1.
Moreover, we demonstrated that Foxm1 directly regulates the expression of VEGF-A expression in prostate tumor cells, suggesting the role of Foxm1 in prostate tumor angiogenesis. Furthermore, our data demonstrated that deletion of Foxm1 from prostate epithelium decreased prostate cancer metastasis in TRAMP mice. Foxm1 transcription factor regulates several genes during prostate cancer progression and metastasis.

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Foxm1 transcription factor, prostate cancer, transgenic mice, epithelial cells

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**PROGRESS REPORT for the period July 2010- June 2011**

## INTRODUCTION

The Forkhead Box M1 (Foxm1) protein is a proliferation specific transcription factor, which is over-expressed in human prostate adenocarcinoma (CaP). Our preliminary studies demonstrated that elevated levels of Foxm1 protein are associated with high proliferation rates in human prostate adenocarcinomas and are directly correlated with the grade of human CaP. Previously, we developed a transgenic mouse line in which Foxm1 is over-expressed in all cell types (Rosa26-Foxm1 mice). These mice were bred with either TRAMP or LADY transgenic (TG) mouse models of prostate cancer. Ubiquitous over-expression of Foxm1 accelerates development of PCa, as well as significantly increases proliferation of prostate tumor cells in both Rosa26-Foxm1/TRAMP and Rosa26-Foxm1/LADY double TG mice. Since Foxm1 is over-expressed in all cell types in Rosa26-Foxm1 mice, the direct role of Foxm1 in prostate epithelial cells, the cells from which prostate cancer arises, remains unknown. The present study seeks to identify **the direct role of Foxm1 in prostate epithelial cells** during initiation and progression of PCa and its potential regulation by $p19^{ARF}$ tumor suppressor. We are testing **two related hypotheses that (1) Foxm1 is essential for the initiation and progression of prostate cancer by regulating genes critical for proliferation of prostate epithelial cells and (2) that Foxm1 is negatively regulated by $p19^{ARF}$ tumor suppressor during PCa formation.** In **aim I** we will determine whether **specific deletion of Foxm1 in prostate epithelial cells *in vivo* diminishes the initiation and progression of PCa.** Moreover, since the $p19^{ARF}$ (human $p14^{ARF}$) gene is frequently inactivated in prostate tumors, in **aim II** we will examine whether **loss of $p19^{ARF}$ function will stimulate the ability of Foxm1 to promote prostate tumor formation.** Completion of these experiments will increase our understanding of the role of Foxm1 in the development of prostate cancer, provide information about molecular mechanisms whereby Foxm1 controls epithelial cell proliferation during prostate tumorigenesis, and determine whether the inhibition of Foxm1 may potentially be beneficial during PCa therapy.

## BODY

### Aim I. Does specific deletion of Foxm1 in prostate epithelial cells diminish the initiation and progression of PCa?

***I-A. To delete Foxm1 from prostate epithelial cells of TRAMP and LADY TG mice.*** We have successfully generated and accumulated the required numbers of prostate epithelial-specific Foxm1 knockout mice (*PB-Cre/Foxm1$^{fl/fl}$*). **(i)** We, first, crossed *PB-Cre* heterozygous male mice with *Foxm1$^{fl/fl}$* homozygous female mice to generate PB-Cre /Foxm1$^{fl/+}$ mice. Then, male *PB-Cre/ Foxm1$^{fl/+}$* mice were crossed with *Foxm1$^{fl/fl}$* females to generate *PB-Cre/Foxm1$^{fl/fl}$* mice. **(ii)** At the same time TRAMP TG mice were crossed with Foxm1$^{fl/fl}$ female mice to generate Foxm1$^{fl/fl}$/TRAMP mice (after 2 generations of back-crossing). After (i) and (ii) were accomplished, the Pb-Cre/Foxm1$^{fl/fl}$ male mice were bred with Foxm1$^{fl/fl}$/TRAMP female mice **(iii)** to generate the Pb-Cre/Foxm1$^{fl/fl}$/TRAMP (epFoxm1$^{-/-}$/ TRAMP) transgenic mice. Experiments with epFoxm1$^{-/-}$/ TRAMP transgenic mice are described below.

After multiple attempts, we were unable to complete breeding of LADY mice (available in my laboratory) with Pb-Cre/Foxm1$^{fl/fl}$mice. It could be due to location of transgene on the same chromosome, or due to unexpected developmental phonotype in triple transgenic mice. We are in the process of acquiring additional breeders for LADY line from Dr. RJ Matusik laboratory. We will use these new breeders during third year of DOD funding. If we will not complete LADY experiment during the third year, we will request for 1 year no cost extension.

***I-B. Effect of epithelial Foxm1 deletion on initiation and progression of prostate carcinogenesis in TRAMP transgenic mice.***

**(i) Epithelial Foxm1 deletion reduced initiation of prostate cancer in TRAMP mice.** To compare initiation of prostate tumorigenesis *epFoxm1$^{-/-}$ /TRAMP* mice, *TRAMP* mice and *Foxm1$^{fl/fl}$/TRAMP* mice were sacrificed at 12 weeks of age. Prior to sacrifice, each mouse was anesthetized and weighed. The genitourinary (GU) tracts consisting of the bladder, urethra, seminal vesicles, ampulary gland, and prostate were excised, weighed and microdissected into individual lobes whenever possible. Paraffin-embedded mouse prostate sections were histologically stained with H&E to evaluate the types of prostatic tumors found in the single and double TG mice as described previously (4). Histological analysis of prostate tissues demonstrated that depletion of FoxM1 from prostate epithelial cells significantly decreased initiation of PINs in *epFoxm1$^{-/-}$ /TRAMP* mice at 12 weeks of age (Table 1). None of the *epFoxm1$^{-/-}$ /TRAMP* mice developed PINs. In contrast, all of the TRAMP single transgenic mice and all of control *Foxm1$^{fl/fl}$/TRAMP* mice developed prostate hyperplasia and PINs at 12 weeks of age (Table 1). This data is consistent with our hypothesis that Foxm1 in tumor cells is essential for initiation of prostate cancer.

**Table 1. Histologic analysis of mouse prostates during initiation of prostate cancer.**

| Mice | % mice with PIN | Number of mice with PIN out of total number |
|---|---|---|
| | 12 weeks of age | |
| *epFoxm1$^{-/-}$ /TRAMP* | 0% | 0/7 |
| *TRAMP* | 100% | 9/9 |
| *Foxm1$^{fl/fl}$/TRAMP* | 100% | 8/8 |

**(ii) Foxm1 was efficiently deleted from prostate epithelium.** To ensure that the conditional deletion of Foxm1 floxed alleles was effectively achieved in prostate epithelial cells, we dissected prostates from control *Foxm1$^{fl/fl}$/TRAMP* and experimental *epFoxm1$^{-/-}$ / TRAMP* male mice to perform quantitative real time RT-PCR for Foxm1 mRNA expression and immunohistochemical staining of prostate paraffin sections with antibodies specific to Foxm1 protein as described (2, 5). Decreased Foxm1 mRNA expression (Fig.1, top panel) and lack of Foxm1 protein in the prostate epithelial cells (Fig.1, bottom panels) indicated a high efficiency of Foxm1 deletion. Thus, Foxm1 was efficiently deleted in prostate epithelial cells of *epFoxm1$^{-/-}$ / TRAMP* mice.

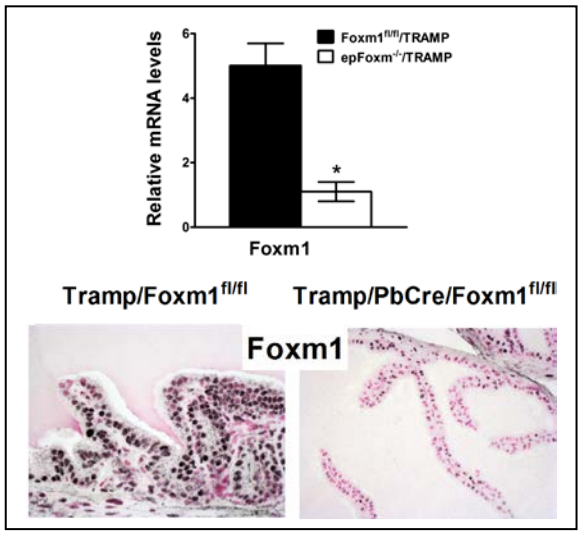


**Figure 1. The efficiency of Foxm1 deletion in prostate epithelium.** Real-time RT-PCR shows decreased levels of Foxm1 mRNA in total RNA prepared from *epFoxm1$^{-/-}$/TRAMP* prostates (top panel). Expression of Foxm1 protein in prostate epithelium (bottom panels). Prostates from *epFoxm1$^{-/-}$ /TRAMP* and control *Foxm1$^{fl/fl}$/TRAMP* mice, were fixed, paraffin-embedded, sectioned, and stained with Foxm1 antibody (dark brown nuclei) and then counterstained with nuclear fast red (red nuclei). Foxm1 expression was observed in prostate epithelial cells of control *Foxm1$^{fl/fl}$/TRAMP* mice. Expression of Foxm1 was dramatically decreased in prostate epithelial cells of *epFoxm1$^{-/-}$/ TRAMP* mice. Magnification is x200.

**(iii) Expression of genes essential for initiation of CaP is decreased in Foxm1 deficient mice.** We are in the process of acquiring this data, using mice described in Fig.1.

***I-C. Role of Foxm1 in progression and metastasis of prostate cancer.***

**(i) Foxm1 deletion from prostate epithelial cells reduced prostate cancer progression.** We hypothesized that epithelial deletion of Foxm1 might directly reduce the progression of prostate cancer by inhibiting DNA replication and/or mitosis in tumor cells. To study the role of Foxm1 on progression of prostate cancer *in vivo*, *epFoxm1*$^{-/-}$ */TRAMP* (n=12), *Foxm1*$^{fl/fl}$*/TRAMP* (n=12), Pb-Cre/ *Foxm1*$^{fl/fl}$ (n=11) and *TRAMP* (n=11, not shown) mice were sacrificed at 20 weeks of age. The genitourinary (GU) tracts consisting of the bladder, urethra, seminal vesicles, and prostate were excised, weighed and microdissected. None of the *epFoxm1*$^{-/-}$ */TRAMP* mice developed prostate carcinomas (CaP). However, 90% of the TRAMP single transgenic mice and 80% of control *Foxm1*$^{fl/fl}$*/TRAMP* mice developed CaP (Table 2). By 20 weeks of age, the weights of the prostate glands from control *Foxm1*$^{fl/fl}$*/TRAMP* and control *TRAMP* mice were 2.3 $\pm$ 1.2 g and 2.7 $\pm$ 2.3 g, respectively. This represents a 20 fold increase compared to *epFoxm1*$^{-/-}$ */TRAMP* mice (Fig. 2). Interestingly, 41.7% of *epFoxm1*$^{-/-}$ */TRAMP* mice developed PINs at 20 weeks of age. Consistent with our hypothesis, Foxm1 deletion from prostate epithelial cells significantly decreased prostate carcinogenesis induced by *TRAMP* (Fig. 2). These results indicate that Foxm1 expression in prostate epithelial cells is required for the progression of prostate cancer in vivo.

**Table 2. Histologic analysis of mouse prostates during progression of prostate cancer**.

| Mice | % mice with PIN | Number of mice with PIN out of total number | % mice with CaP | Number of mice with CaP out of total number |
|---|---|---|---|---|
| | | | 20 weeks of age | |
| *epFoxm1*$^{-/-}$*/TRAMP* | 41.7% | 5/12 | 0% | 0/12 |
| *TRAMP* | 10% | 1/10 | 90% | 9/10 |
| *Foxm1*$^{fl/fl}$*/TRAMP* | 20% | 2/10 | 80% | 8/10 |
| | | | | |

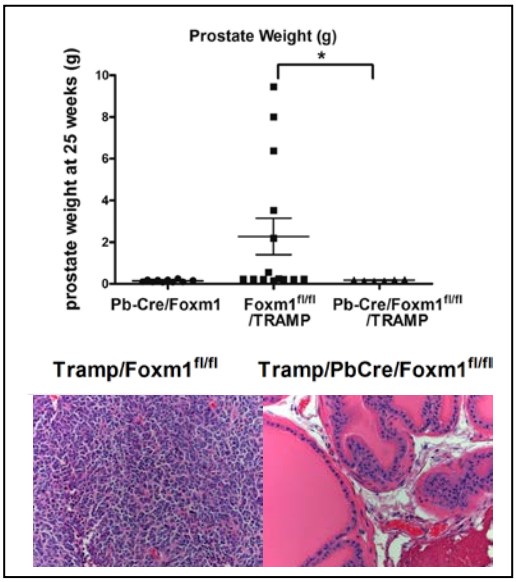


**Figure 2. Foxm1 deletion from prostate epithelial cells reduced prostate carcinogenesis.** Experimental *epFoxm1*$^{-/-}$*/TRAMP* and control mice were sacrificed at 25 weeks of age. Prostate glands were collected and weighed from experimental *epFoxm1*$^{-/-}$*/TRAMP* (n=10), control *Foxm1*$^{fl/fl}$*/TRAMP* (n=12) and control *Pb-Cre/ Foxm1*$^{fl/fl}$ (n=11) mice. Deletion of *Foxm1* significantly decreased ($p < 0.05$) the weight of prostate glands at 25 weeks of age (top panel). Mean weights of prostate glands (±SD) were calculated from 10-12 mouse prostates per group. A *p* value < 0.05 is shown with asterisk (*). Using H&E staining (bottom panels), prostate adenocarcinomas were identified in *Foxm1*$^{fl/fl}$*/TRAMP* mice (left bottom panel) and PINs in *epFoxm1*$^{-/-}$*/TRAMP* prostate (right bottom panel).

**(ii). Foxm1 deletion from prostate epithelial cells diminished tumor cell proliferation.** Decreased carcinogenesis in *epFoxm1*$^{-/-}$*/ TRAMP* mice was associated with decreased proliferation of tumor cells and reduced expression of cell cycle regulatory genes *Cdc25b*, *Cyclin B1*, and *Plk-1* (Fig. 3 and Fig. 4). Cell proliferation was determined by the immunochistochemical detection of Ki-67, which is a proliferation specific marker. To detect prostate tumor cells undergoing mitosis, we used antibodies against phospho-Histone H3 (pH3), specific marker for cells undergoing mitosis. We did not observe any differences in the number of apoptotic cells in the tumors from control and *epFoxm1*$^{-/-}$*/ TRAMP* mice (data not shown).

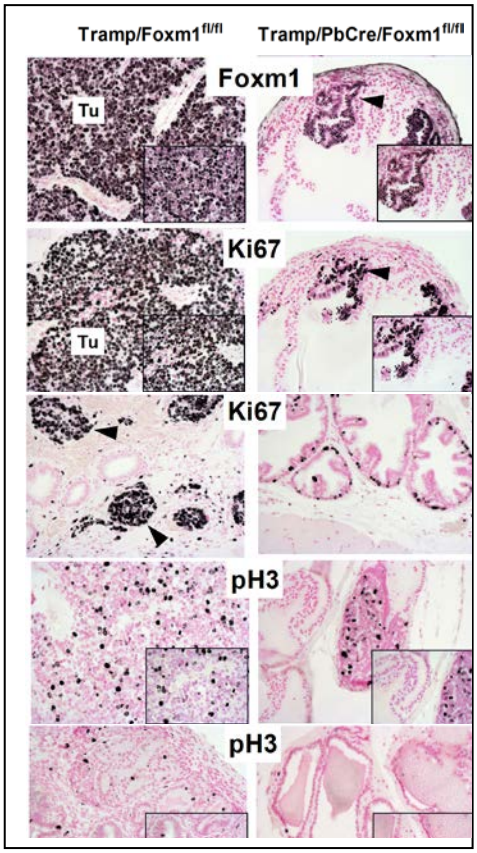


**Figure 3. Foxm1 deletion from prostate epithelial cells diminished tumor cell proliferation.** Mouse prostate glands were harvested 25 weeks after birth and used for immunohistochemistry with antibodies against Foxm1, KI-67 and PH3 as described. Decreased proliferation of tumor (Tu) cells was observed in Foxm1-deficient prostates as demonstrated by reduced numbers of Ki-67-positive and PH3-positive tumor cells in *epFoxm1*$^{-/-}$*/TRAMP* prostate glands compared to control mice. Magnification is x100.

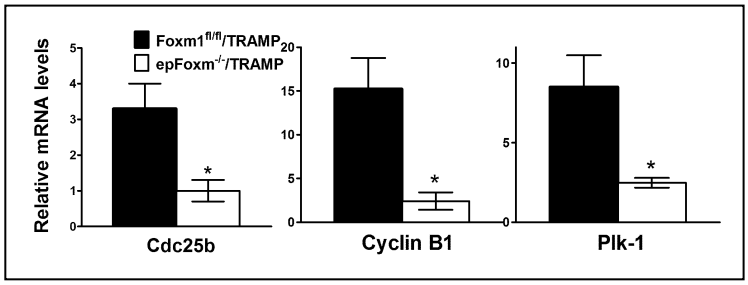


**Figure 4. Foxm1 is essential for proliferation of prostate cancer cells by regulating genes critical for cell cycle progression.** Decreased mRNA levels of *Cdc25b, Cyclin B1* and *Plk-1* in *epFoxm1*$^{-/-}$*/TRAMP* prostates was demonstrated by qRT-PCR. b-actin mRNA was used for normalization. A p value <0.05 is shown with (*).

**(iii). Role of Foxm1 in tumor metastasis.** To study the role of Foxm1 on metastasis of prostate carcinomas, experimental *epFoxm1*$^{-/-}$*/TRAMP* (n=10) and control *Foxm1*$^{fl/fl}$*/TRAMP* (n=12) mice were sacrificed at 25 weeks of age. Prostate glands were collected and weighed. All pelvic lymph nodes were harvested. To detect pulmonary metastasis, the lungs of each mouse were inflated with 4% paraformaldehyde for histological analysis. Grossly evident metastatic lesions were harvested and fixed for histological analysis. Total numbers of metastatic lesions were counted in each mouse. Deletion of *Foxm1* significantly decreased ($p < 0.05$) the

number of mice with the metastasis (Figure 7). We continue these experiments to identify molecular mechanisms regulated by Foxm1 in tumor metastasis.

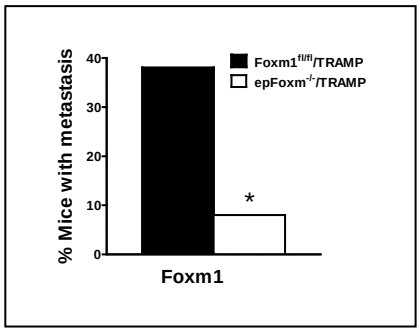


**Figure 7. Deletion of Foxm1 from prostate epithelium in TRAMP mice decreased prostate cancer metastasis.** A *p* value < 0.05 is shown with asterisk (*).

**(iv) Expression of genes essential for progression of CaP is decreased in Foxm1 deficient mice.** Since angiogenesis plays an essential role in prostate cancer progression and VEGF-A is a main angiogenic factor produced by tumor cells, we compared the levels of *VEGF-A* mRNA in *epFoxm1$^{-/-}$/TRAMP* and control mice prostates. Mouse prostate glands were harvested 25 weeks after birth and used to isolate total prostate mRNA. Using real time RT-PCR, we demonstrated that mRNA levels of *VEGF-A* in *epFoxm1$^{-/-}$/TRAMP* prostates were significantly decreased (Fig. 5). Thus, Foxm1 in prostate tumor cells is critical for *VEGF-A* expression during cancer progression.

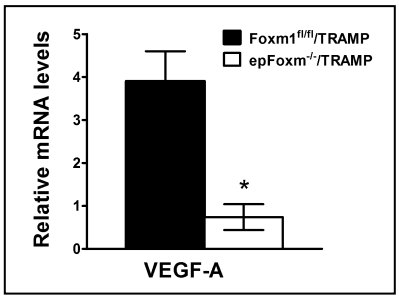


**Figure 5. Deletion of Foxm1 from prostate epithelium decreased VEGF levels during PCa.** Mouse prostate glands were harvested 25 weeks after birth and used to isolate total prostate mRNA. Decreased mRNA levels of *VEGF* in *epFoxm1$^{-/-}$/TRAMP* prostates was demonstrated by qRT-PCR. b-actin mRNA was used for normalization. A p value <0.05 is shown with (*).

Decreased expression of *VEGF-A* in *epFoxm1$^{-/-}$/TRAMP* prostates suggested that this genes is a transcriptional target of Foxm1. To determine whether Foxm1 regulates expression of *VEGF-A* gene *in vitro*, U2OS human cells were transfected with short interfering RNA (siRNA) specific to the human *Foxm1* mRNA (siFoxm1) or with mutant siFoxm1, containing 5 mutations in recognition sequence (2). Seventy-two hours after siRNA transfection, total RNA was prepared from the U2OS cells and analyzed for *Foxm1* expression by qRT-PCR. siFoxm1 transfection efficiently reduced *Foxm1* mRNA (Fig. 6A). Consistent with our *in vivo* studies (Fig. 5), Foxm1-depletion in cultured tumor cells significantly decreased *VEGF-A* mRNA.

Since Foxm1 deficiency was associated with decreased *VEGF-A* expression *in vivo* and *in vitro* (Fig. 5 and 6A), we investigated whether Foxm1 transcriptionally activated promoter regions of *VEGF-A* gene. The potential Foxm1 DNA binding sites were identified in the -2.3Kb promoter region of the human *VEGF-A* gene. To determine whether the Foxm1-binding sites were transcriptionally active, co-transfection experiments were performed using CMV-Foxm1b expression vector (1, 3) and luciferase (LUC) reporter construct driven by -2.3Kb *VEGF-A* promoter regions. Co-transfection of the CMV-Foxm1b expression vector significantly increased activity of the reporter when compared to CMV-empty vector (Fig. 6B). These results demonstrated that Foxm1 transcriptionally activated the human *VEGF-A* promoter region, suggesting that *VEGF-A* gene is a direct Foxm1 target.

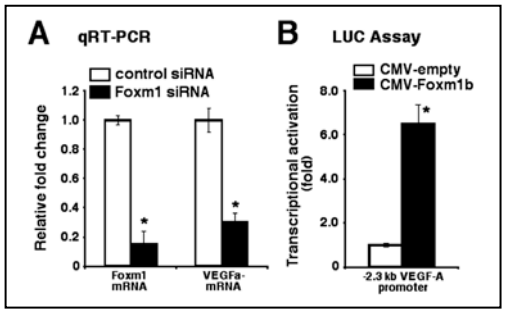


**Figure 6. VEGF is a direct target of Foxm1 transcription factor. A.** Foxm1 depletion in A549 human adenocarcinoma cells reduced *VEGFa* mRNA expression. A549 cells were mock transfected (control siRNA) or transfected with short interfering RNA (siRNA) duplex specific for *Foxm1* mRNA (Foxm1 siRNA). Human b-actin mRNA was used for normalization. **B.** *VEGFa* is a direct transcriptional target of Foxm1. Luciferase assay demonstrated that Foxm1 induced the transcriptional activity of *VEGFa* promoter. U2OS cells were transfected with CMV-Foxm1b expression vector and luciferase (LUC) reporter driven by -2.3kb human *VEGFa* promoter region. CMV-empty plasmid was used as a negative control. Cells were harvested at 24 hr after transfection and processed for dual LUC assays to determine LUC activity. Transcriptional activity of the human *VEGFa* promoter was increased by CMV-Foxm1b transfection. A $p$ value < 0.05 is shown with asterisk (*).

To identify potential Foxm1 targets, expression of several genes critical for prostate cancer progression and metastasis was examined. Total RNA was isolated from excised prostate glands. Using real time RT-PCR analysis, we demonstrated that several genes were significantly down-regulated in *epFoxm1$^{-/-}$/TRAMP* prostates (Fig. 8). Our results suggest that reduced expression of these genes can contribute to reduced tumor metastasis in Foxm1-defecient mice.

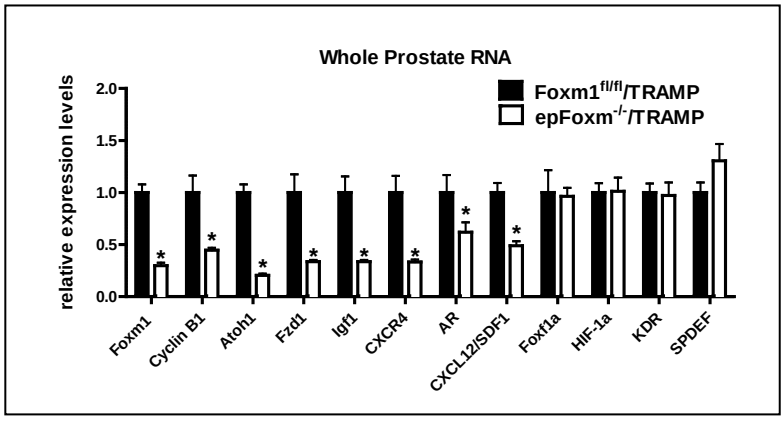


**Figure 8. Foxm1 transcription factor regulates epithelial genes critical for prostate cancer progression and metastasis.** qRT-PCR shows reduced mRNA levels of Atoh1, Fzd1, Igf1, CXCR4, AR and CXCL12 in *epFoxm1$^{-/-}$/TRAMP* prostates.

**<u>Aim II. Does deletion of p19$^{ARF}$ tumor suppressor promote Foxm1-mediated prostate carcinogenesis?</u>**

***II-A. To generate Pb-Foxm1 and Pb-Foxm1/TRAMP transgenic mice.*** To address this specific aim we already generated mice with Dox-inducible Foxm1 expression. During the second year of DOD funding, we bred TetO-GFP-FoxM1-ΔN transgenic mice with TRAMP transgenic mice to generate double transgenic mice (*TetO-GFP-FoxM1-ΔN/ TRAMP*). These double transgenic mice were bred with *Pb-Cre$^{tg/+}$/ LoxP-stop-LoxP-rtTA(Rosa26)$^{tg/tg}$* mice, the latter of which contained a reverse tetracycline activator (rtTA) knocked into Rosa26 locus. In Pb-Cre$^{tg/+}$/ LoxP-stop-LoxP-rtTA(Rosa26)$^{tg/tg}$ mice, Dox treatment results in prostate epithelial-specific expression of rtTA due to excision of LoxP-stop-LoxP cassette by the Cre recombinase. After all these breeding, Pb-Cre$^{tg/+}$/ LoxP-stop-LoxP-rtTA(Rosa26)$^{tg/+}$ / TetO-GFP-FoxM1-ΔN $^{tg/tg}$ / TRAMP transgenic mice were generated (we abbreviated this mouse line as *Pb-Foxm1-ΔN / TRAMP* mice). Transgenic mice Pb-Cre$^{tg/+}$/ LoxP-stop-LoxP-rtTA(Rosa26)$^{tg/+}$ / TetO-GFP-FoxM1-ΔN $^{tg/tg}$ were abbreviated as *Pb-Foxm1-ΔN* ***In these mice the over expression of Foxm1 in prostate epithelium is inducible by Dox which allow us to study the role of Foxm1 depending on the stage of cancer.***

***II-B. To determine whether Foxm1 expression in prostate epithelium will stimulate prostate tumorigenesis.*** In our pilot experiment*, Pb-Foxm1-ΔN* (n=4) mice were sacrificed at 15 weeks of age. The genitourinary (GU) tract consisting of the bladder, urethra, seminal vesicles, and prostate were excised, weighed and microdissected. Our pilot experiment with 7 mice demonstrated that *Pb-Foxm1-ΔN* mice

developed PINs at 15 weeks of age (Table 3), suggesting that over-expression of Foxm1 in prostate epithelial cells causes epithelial hyperplasia and PIN formation. We are in the process of accumulating additional numbers of triple transgenic mice to increase statistical power of our experiments. 22 additional mice were generated and treated with Dox. These mice will be sacrificed in November-December to examine prostate tumors.

**Table 3. Histologic analysis of mouse prostates during progression of prostate cancer.** Over-expression of Foxm1 in prostate epithelial cells caused PIN formation, but did not progress to carcinomas by 15 weeks of age. No PINs were found in control mice at this time point.

| | % mice with PIN | Number of mice with PIN out of total number |
|---|---|---|
| *Pb-Foxm1-ΔN* | 75% | 3/4 |
| *Control (Pb-Cre)* | 0% | 0/3 |
| | | |

***II-C. To determine whether loss of p19$^{ARF}$ tumor suppressor will increase Foxm1 dependent prostate carcinogenesis.*** Since p19$^{ARF}$ protein inhibits Foxm1 transcriptional activity during development of hepatocellular carcinoma, we studied the role of p19$^{ARF}$ in prostate tumorigenesis. For this purpose, we bred the *Pb-Foxm1-ΔN* mice TG mice with *p19$^{ARF\ +/-}$* mice to generate *Pb-Foxm1-ΔN /p19$^{ARF\ +/-}$* mice. It takes 25 weeks for the prostate cancer to develop. We will harvest the first mice in one month. These groups consist of sixteen *Pb-Foxm1-ΔN /p19$^{ARF\ +/-}$* quadruple transgenic mice and sixteen control mice.

## KEY RESEARCH ACCOMPLISHMENTS

- Foxm1 deletion from prostate epithelial cells decreased initiation and progression of prostate cancer in TRAMP mice.
- Foxm1 deletion from prostate epithelial cells diminished tumor cell proliferation.
- Foxm1 is essential for proliferation of prostate cancer cells by regulating genes critical for cell cycle progression.
- Deletion of Foxm1 from prostate epithelium or cultured tumor cells decreased VEGF expression. VEGF is a direct target of Foxm1 transcription factor.
- Deletion of Foxm1 from prostate epithelium in TRAMP mice decreased prostate cancer metastasis.
- New transgenic mice with conditional over-expression of Foxm1 in prostate epithelial cells (*Pb-Foxm1-ΔN*) were successfully generated. Pilot experiment demonstrated that over-expression of Foxm1 in prostate epithelial cells caused PIN formation, but did not progress to carcinomas by 15 weeks of age.
- The new transgenic *Pb-Foxm1-ΔN* mice were successfully crossed with *p19$^{ARF\ +/-}$* mice and *Pb-Foxm1-ΔN* /p19$^{ARF\ +/-}$ mice were generated.

## REPORTABLE OUTCOMES

- The employment of Emily Cross, a research assistant, was supported by this award.
- Data was presented at;
    - 2011 IMACT meeting (March, 2011, Orlando, FL). Abstract: Balli D., Cross E., Snyder J., Zhang Y. and Kalin T.V., "Role of Foxm1 Protein in Pathogenesis of Prostate Cancer"
    - 2011 Cincinnati Cancer Symposium on NF-kB, Cancer and Inflammation (May, 2011, Cincinnati, OH). Abstract: Balli D., Cross E., Snyder J., Zhang Y. and Kalin T.V., "Dissecting the Role of Foxm1 in Prostate Cancer". Presented by the graduate student David Balli.
    - 2011 Presentation at the Summer Undergraduate Research Fellowship (SURF) Symposium (August, 2011, University of Cincinnati, Cincinnati, OH). Talk title: "Role of Foxm1 Protein in Initiation and Progression of Prostate Cancer". Presented by the undergraduate summer student Andrea Hiller.
- We are currently preparing manuscript on the role of Foxm1 in prostate epithelial cells during development of prostate cancer. Manuscript will be submitted to Cancer Research.

## CONCLUSIONS

- Pb-Cre efficiently deleted *Foxm1* allele from prostate epithelium in *PB-Cre/Foxm1*$^{fl/fl}$ mice.

- Foxm1 deletion from prostate epithelial cells decreased initiation and progression of prostate cancer in TRAMP mice by regulating proliferation of tumor cells.

- Foxm1 directly targets VEGF, which is a key player in tumor angiogenesis.

- Deletion of Foxm1 from prostate epithelium in TRAMP mice decreased prostate cancer metastasis.

- Inhibition of Foxm1 may potentially be beneficial during PCa therapy.

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**APPENDICES.**

1. 2011 IMACT meeting (March, 2011, Orlando, FL). Poster: Balli D., Cross E., Snyder J., Zhang Y. and Kalin T.V., "**Role of Foxm1 Protein in Pathogenesis of Prostate Cancer**"


Abstract.

***Background and Objectives.*** The Forkhead Box M1 (Foxm1) protein is a proliferation specific transcription factor and is over-expressed in human prostate adenocarcinoma. We demonstrated that elevated levels of Foxm1 protein are associated with high proliferation rates in human prostate adenocarcinomas and are directly correlated with the grade of human prostate cancer (PCa). Ubiquitous over-expression of Foxm1 in all cell types accelerates development of PCa, as well as significantly promotes proliferation of prostate tumor cells in transgenic mice. However, the direct role of Foxm1 in prostate epithelial cells, the cells from which prostate cancer arises, remains unknown. The present study seeks to identify **the direct role of Foxm1 in prostate epithelial cells** during PCa and its potential regulation by $p19^{ARF}$ tumor suppressor. We are testing two related hypotheses that **(I) Foxm1 is essential for the initiation and progression of prostate cancer by regulating genes critical for proliferation of prostate epithelial cells** and **(II) that Foxm1 is negatively regulated by $p19^{ARF}$ tumor suppressor during PCa formation.**

***Methodologies.*** In **aim I** we are determining whether **specific deletion of Foxm1 in prostate epithelial cells *in vivo* diminishes the initiation and progression of PCa.** Moreover, since the $p19^{ARF}$ (human $p14^{ARF}$ ) gene is frequently inactivated in prostate tumors, in **aim II** we will examine whether **loss of $p19^{ARF}$ function will stimulate the ability of Foxm1 to promote prostate tumor formation.**

***Results to data.*** **Aim I.** During the first year of grant support, we have successfully generated the prostate epithelial-specific Foxm1 knockout mice (*PB-Cre/Foxm1$^{fl/fl}$*). The Pb-Cre/Foxm1$^{fl/fl}$ male mice were bred with Foxm1$^{fl/fl}$/TRAMP female mice to generate the Pb-Cre/Foxm1$^{fl/fl}$/TRAMP (epFoxm1$^{-/-}$/ TRAMP) transgenic mice. In these mice Foxm1 is deleted from prostate epithelium. The efficiency of Foxm1 deletion was evaluated by quantitative real time RT-PCR and immunohistochemical staining of prostate paraffin sections with antibodies specific to Foxm1. These studies demonstrated that Foxm1 was efficiently deleted in prostate epithelial cells in *epFoxm1$^{-/-}$ / TRAMP* mice.

In our pilot experiment*, epFoxm1$^{-/-}$ /TRAMP* (n=2), *TRAMP* (n=3) and *Foxm1$^{fl/fl}$/TRAMP* (n=2) mice were sacrificed at 12 weeks of age. PINs were observed in *TRAMP* (3 mice with PINs out of 3) and *Foxm1$^{fl/fl}$/TRAMP* (2 mice with PINs out of 2) mice. However, we did not find any PINs in *epFoxm1$^{-/-}$ /TRAMP* mice (0 mice with PINs out of 2), suggesting that Foxm1 expression in prostate epithelial cells is required for the initiation of prostate carcinogenesis *in* TRAMP mice. We are in the process of generating additional mice to confirm our conclusion.

**Aim II.** We are inducing Foxm1 expression in the prostate epithelial cells by generating mice with Foxm1 transgene under control of the Pb promoter (Pb-Foxm1 mice). We will breed these mice with TRAMP and LADY TG mice to generate Pb-Foxm1/TRAMP and Pb-Foxm1/LADY TG mice to examine whether over-expression of Foxm1 in prostate epithelial cells accelerates the formation of prostate tumors. Furthermore, the Foxm1 transgenic mice will be bred into a $p19^{ARF-/-}$ genetic background to determine whether the effect of transgenic Foxm1 protein on prostate tumorigenesis is enhanced in the absence of $p19^{ARF}$.

Three TetO-GFP-FoxM1-ΔN transgenic mouse lines were established and showed similar phenotypes. We started the breeding of TetO-GFP-FoxM1-ΔN transgenic mice with TRAMP transgenic mice to generate double transgenic mice (*TetO-GFP-FoxM1-ΔN/ TRAMP*).

***Conclusions.*** New transgenic mice with conditional deletion of Foxm1 from prostate epithelial cells (*PB-Cre/Foxm1$^{fl/fl}$*) were successfully generated. The efficient Foxm1 deletion was shown by qRT-PCR and immunostaining. The new transgenic *PB-Cre/Foxm1$^{fl/fl}$* mice were successfully crossed with TRAMP mice (epFoxm1$^{-/-}$/ TRAMP). Foxm1 deletion from prostate epithelial cells decreased prostate carcinogenesis in these mice. Construct and mice with Dox-inducible Foxm1 expression were successfully generated.

***Impact.*** Completion of these experiments will increase our understanding of the role of Foxm1 in the development of prostate cancer, provide information about molecular mechanisms whereby Foxm1 controls

epithelial cell proliferation during prostate tumorigenesis, and determine whether the inhibition of Foxm1 may potentially be beneficial during PCa therapy, therefore leading to the development of the novel therapeutic target for prostate cancer chemotherapy.

**2.** 2011 Cincinnati Cancer Symposium on NF-kB, Cancer and Inflammation (May, 2011, Cincinnati, OH). Balli D., Cross E., Snyder J., Zhang Y. and Kalin T.V., Talk title: “Dissecting the Role of Foxm1 in Prostate Cancer”. Presented by the graduate student David Balli.

**3.** 2011 Summer Undergraduate Research Fellowship (SURF) Program Retreat (August, 2011, Cincinnati, OH). Andrea Hiller, Balli D., Cross E., Snyder J., Zhang Y. and Kalin T.V., Talk title: “Role of Foxm1 in Prostate Cancer Progression and Metastasis”. Presented by the undergraduate student Andrea Hiller.